N-Sulfanilyl-1-alkylcytosines.[1] A New Highly Active Class of "Soluble," Short-Acting Sulfanilamides

LEONARD DOUB, ULDIS KROLLS, J. M. VANDENBELT,

The Department of Chemistry, Division of Medical and Scientific Affairs, Parke, Davis and Co., Ann Arbor, Michigan 48106

AND M. W. FISHER

The Department of Biological Research and Development, Division of Medical and Scientific Affairs, Parke, Davis and Co., Detroit, Michigan 48232

Received August 15, 1969

N^4-Sulfanilylcytosine **8a** and nine 1-alkyl derivatives **8b–8j** were prepared. The parent **8a** was not active but all derivatives were highly active in experimental mouse infections with *Staphylococcus aureus*, *Proteus vulgaris*, and *Escherichia coli*. The compound showing highest activity and high solubility was the 1-ethyl derivative **8c**. Tests *in vivo* indicate that **8c** is a quickly absorbed, quickly excreted (short acting) sulfa drug potentially and especially suitable for the treatment of urinary tract infections. In systemic experimental mouse infections **8c** is substantially more active than certain other short-acting, soluble urinary tract sulfanilamides such as sulfisoxazole, sulfamethizole, and sulfisomidine. Calculating from the binding to human serum albumin **8c** should give as high or higher "free" levels and thus promise equal or greater activity against the tissue component of human urinary infections. The potential freedom of **8c** from crystalluria reactions is indicated by its high equilibrium solubility (pH 5–5.5) and by its tendency to form supersaturated solutions in urine of extraordinary stability. The concentration of such solutions can exceed the true solubility many fold.

We have prepared a number of sulfanilylcytosines **8** (Scheme I) and have found them to be active as antibacterials, particularly in experimental infections induced with Gram-positive and Gram-negative pathogenic species.

We synthesized these derivatives by the methods shown in Scheme I.[2] The beginning 4-thiouracils **2** are available by thiation of uracil (**1a**), or by thiation of the appropriate 1-substituted uracil **1b–j** available by the methods of Shaw.[3] To the extent that these procedures begin with expensive uracils, the cytosines they yield are necessarily also expensive. We will present in later papers some new departures in cytosine syntheses aimed at making them available more economically.

The new sulfanilylcytosines were tested comparatively in acute infections, in mice treated by a single oral dose at the time of challenge in a standardized procedure.[4] The ED_{50} values for these derivatives in various experimental infections are given in Table I. Listed also in Table I are the solubilities of the new compounds in pH 5 buffer at room temperature.

The parent compound **8a** is inactive in all tests and is the only inactive one of the group. Each of the remaining compounds of Table I is highly active. Only two compounds (**8a** and **8i**) are poorly soluble and they are the only compounds of Table I which clearly lie below the preferred solubility limit of 50 mg/100 ml (at pH 5–5.5). This is our transposition to room temperature of the 37° solubility limit proposed by Lehr, above which he suggested that the likelihood of kidney blockade by precipitation is minimal (70 mg/100 ml).[5] The ethyl derivative **8c** has a solubility compatible with Lehr's limit. Since solubility is good and activity is maximal in this derivative it was selected for further evaluation. Now with the name sulfacytine,[6] **8c** has been studied intensively in our laboratories; most of this work will be reported by others elsewhere. We present here some of the data which were used originally to define our interest in the compound.

Sulfacytine (**8c**) from its behavior in mice appears to be absorbed and excreted rapidly, *i.e.*, short acting. This is illustrated in Table II where **8c** is compared with the poorly soluble but highly active sulfadiazine. The infections denoted in Table II are quickly lethal and we infer that the great drop in effectiveness when comparing early and late treatment reflects a prompt elimination of the drug, the somewhat more persistent sulfadiazine being less affected.[7] When **8c** is tested as a single oral dose before challenge against a much more slowly developing infection (*Streptoccus pyogenes*, not reported here) it is essentially inactive ($ED_{50} > 500$ mg/kg). However if the drug is administered in the diet throughout the course of this streptococcal infection it displays distinct activity ($ED_{50} = 32$ mg/kg). We take this as additional evidence that sulfacytine is of the short acting type.

It was of interest to compare sulfacytine (**8c**) with certain prominent short-acting drugs. This comparison is detailed in Table III, wherein four compounds were tested in mice against acute lethal infections involving seven bacterial species and 12 strains representative of the more commonly encountered Gram-negative urinary pathogens. A general comparison of efficacy among all four compounds, as indicated by the ED_{50} values in Table III, consistently verifies the greater intrinsic antibacterial potency of sulfacytine. On a quantitative basis, derived from geometric means (GM) of ED_{50}'s for each drug, sulfacytine's GM ED_{50} 12 mg/kg classed it as about 3.5 times more potent than sulfisoxazole (GM, ED_{50} 43 mg/kg) and sulfisomidine (GM, ED_{50} 40 mg/kg), and about 18 times more potent than sulfamethizole (GM, ED_{50} 220 mg/kg).

(1) This name brings out cytosine as a class feature. The *Chemical Abstracts* name is N^1-(1-alkyl-1,2-dihydro-2-oxo-4-pyrimidinyl)sulfanilamide.

(2) L. Doub and U. Krolls, U. S. Patent 3,375,247 (1968).

(3) G. Shaw, *J. Chem. Soc.*, 1834 (1955).

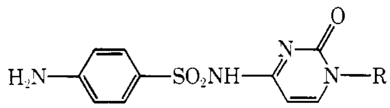
(4) A. L. Erlandson, M. W. Fisher, L. A. Gagliardi, and M. R. Gaetz, *Antibiot. Chemother.*, **10**, 84 (1960).

(5) D. Lehr, *Ann. N. Y. Acad. Sci.*, **69**, 417 (1958).

(6) Accepted by the USAN Committee as the generic name for 1-ethyl-N^4-sulfanilylcytosine: N^1-(1-ethyl-1,2-dihydro-2-oxo-4-pyrimidinyl)sulfanilamide.

(7) This kind of interpretation has been used by others previously, see, for example: G. S. Redin and M. E. McCoy, *Chemotherapia*, **4**, 386 (1962).

TABLE I
SULFANILYLCYTOSINES



No.	R	Mp, °C	Formula[a]	Mg/kg oral ED$_{50}$[b] S. aureus	P. vulgaris	E. coli	Solubility,[c] pH 5, 25°, mg/100 ml
8a	H	286–287 dec	$C_{10}H_{10}N_4O_3S$	>250	>250	>250	7.5
8b	Me	219–222	$C_{11}H_{12}N_4O_3S$	85	55	23	122
8c	Et	167–168	$C_{12}H_{14}N_4O_3S$	12–23	3.2–5.3	4.1–5.0	109
	·H$_2$O	104	$C_{12}H_{16}N_4O_4S$[d]				51
8d	Pr	141–142	$C_{13}H_{16}N_4O_3S$	20	6.2	8	32
8e	*i*-Pr	201–203	$C_{13}H_{14}N_4O_3S$	11	2.3	6.5	21
8f	Bu	118–120	$C_{14}H_{18}N_4O_3S$[e]	33	9	17.5	26
8g	*i*-Bu	133–135	$C_{14}H_{18}N_4O_3S$	35	10.5	20	25
8h	*sec*-Bu	136–138	$C_{14}H_{18}N_4O_3S$	28	9.5	13	56
8i	PhCH$_2$	207–208	$C_{17}H_{16}N_4O_3S$	10	5.5	90	0.56
8j	Allyl	165–167	$C_{13}H_{14}N_4O_3S$	33	3.1	5.5	24
Sulfisoxazole				90	14	35	37

[a] All compounds were analyzed for C, H, N, S. [b] Derived from results with single oral doses given at time of challenge with *Staphylococcus aureus* (UC-76), or *Proteus vulgaris* (UC-232), or *Escherichia coli* (055B5). [c] Determined in the presence of a large excess of undissolved drug: see Experimental Section. [d] Also H$_2$O determination. [e] Analysis corrected for 1.06% water.

TABLE II
ACTIVITY AND DOSE TIMING

Organism	Time of single oral dose	Approximate value for 50% Effective dose (ED$_{50}$), mg/kg: Sulfadiazine	Sulfacytine
S. aureus UC 76	6-hr prechallenge	57	95
	At challenge	26	23
E. coli 055:B5	6-hr prechallenge	5.5	16.5
	At challenge	2.9	5.0
P. vulgaris UC 232	6-hr prechallenge	2.7	13.5
	At challenge	1.9	3.3

TABLE III
COMPARISON WITH SHORT ACTING SULFONAMIDES

Challenge organism	Approximate ED$_{50}$, mg/kg[a] Sulfacytine	Sulfisoxazole	Sulfamethizole	Sulfisomidine
A. aerogenes (Marshall)	12	55		
E. coli (MGH-1)	5	25	125	25
E. coli (Vogel)	5	23		
E. coli (075)	13	20		
E. coli (055B5)	5	35		
P. mirabilis (MGH-1)	4	16	160	10
P. mirabilis (RC-2247)	10	25		
P. morganii (RC 2362)	15	35		
P. vulgaris (1810)	4	16	140	15
P. vulgaris (UC 232)	7	21		
Pseudomonas aeruginosa (28)	220	520	850	675
P. aeruginosa (F-58)	65	410		
S. aureus (UC-76)	23	90		

[a] Single oral dose given at time of challenge; data are average from replicate tests, and an approximate ±50% variation applies in most instances. Challenges were intraperitoneal with an esimated 100 LD$_{50}$ for each strain.

In human therapy a "soluble" short-acting sulfanilamide drug would be used preferably in acute urinary tract disease because it would promptly afford high urine levels of drug. However, the blood (and tissue) levels of the drug may in certain circumstances also be important. To the extent that they are, and since these sulfonamides are adsorbed extensively to blood protein, it would be the "free" unbound level which may be effective antibacterially. In Table IV is shown the binding to 4% serum albumin of sulfacytine, sulfisoxazole, sulfamethizole, and sulfisomidine. Albumin usually accounts for most of the binding of drugs to blood protein; it also occurs in serum at the 4% concentration used here.[8,9] We therefore feel that the data of Table IV concerning human serum albumin should be approximately predictive, with each sulfanilamide, for the relative ratios of free to total drug which would be encountered in human therapy.

TABLE IV
BINDING TO 4% SERUM ALBUMIN AT pH 7, ACCOMPANYING A THERAPEUTIC "FREE" (UNBOUND) LEVEL OF DRUG (1 mg/100 ml)[a]

	Human Fraction V: % binding (% free)	Total drug concn (mg/100 ml)	Ratio total X: total 8c	Human crystn % binding	Bovine fract V, % binding
Sulfacytine (8c)	86 (14)	7	1	86.5	93.5
Sulfisoxazole	92 (8)	13	2	93	93
Sulfamethizole	93 (7)	14	2	93	92
Sulfisomidine	86 (14)	7	1	86.5	

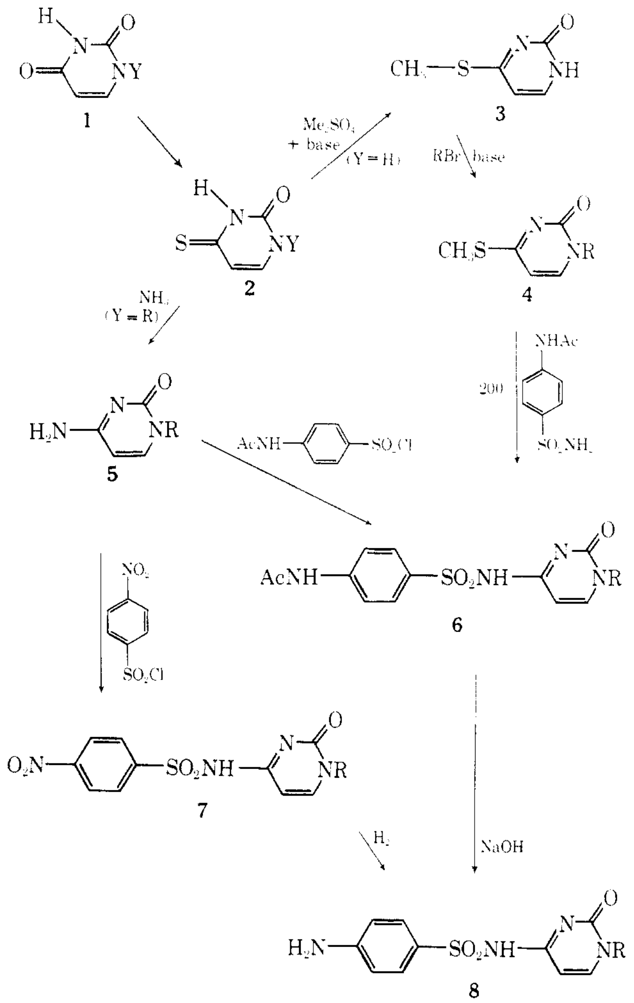
[a] Percentage binding approaches its maximum as the free (and total) level of drug approaches zero; conversely, percentage binding decreases with increasing free (and total) level of drug. This free level was chosen because it probably relates to a therapeutic level; see text.

The binding adsorptions of these drugs to human fraction V and crystalline albumin shown in the first and fourth columns, respectively, of Table IV fall into a narrow range well within that observed for many sulfanilamide drugs.[8] However, the apparently small differences in total bound drug become large differences if referred to there maining small "free" percentages,

(8) R. E. Bagdon in "Experimental Chemotherapy," Vol. II, R. J. Schnitzer and F. Hawking, Ed., Academic Press, New York, N. Y., 1964, pp 290–292.

(9) C. H. Best and N. B. Taylor, "The Physiological Basis of Medical Practice," 7th ed, The Williams Wilkins Co., Baltimore, Md., 1961, p 5.

Scheme I



a, R = H f, R = Bu
b, R = Me g, R = *i*-Bu
c, R = Et h, R = *sec*-Bu
d, R = Pr i, R = benzyl
e, R = *i*-Pr j, R = allyl

which are given in parentheses in the first column. The significance of these differences is more directly apparent from the data of column 2 listing the total concentration of drug in mg/100 ml which must be attained in order to achieve 1 mg/100 ml of "free" (active) drug. Sulfisoxazole and sulfamethizole are about equal and require a total level of drug roughly twice that needed by sulfacytine or sulfisomidine to reach the 1 mg/100 ml free level (column 3).

The 1 mg/100 ml "free" level was chosen because it corresponds roughly to the portion of unbound drug in the 5–15 mg/100 ml of total drug serum concentration which in the early days of sulfanilamide therapy was set as a desirable therapeutic level for the treatment of major systemic disease.[10] This high serum level came from a daily dose of about 6 g. It is likely that the quickly excreted, short-acting sulfanilamides used at the lower dosages (2 g and as low as 0.5 g/day) usually employed in urinary tract infection give distinctly lower total serum concentrations.[11] To the extent that this is true the superiority of **8c** and sulfisomidine becomes even more pronounced. For instance, at the *total* serum concentration of 1 mg/100 ml (in contrast to the 5–15 mg/100 ml cited above) the free drug left unbound by albumin is about three times greater for **8c** than for sulfisoxazole or sulfamethizole (0.09, 0.03, 0.03 mg/100 ml, respectively).[12]

It is worth noting that no direct extrapolation for these sulfanilamides can be made from the binding by the serum albumin of one species to the binding by the albumin of another. This is illustrated in the last column of Table IV. The binding by bovine serum albumin compared to that by human serum albumin increases for sulfacytine, and is about the same for sulfamethizole and for sulfisoxazole. This kind of selectivity among proteins is not unique but is probably another instance of specificity in protein binding; see Bagdon[8] for a summary.

The equilibrium solubility of **8c** in pH 5 buffer at 37° is as follows: anhydrous (at pseudoequilibrium) = approximately 175 mg/100 ml; hydrate = 105 mg/100 ml. This solubility is higher than the room temperature values given in Table I, which, as previously mentioned, were already adequate by the solubility safety standards proposed by Lehr (*ca.* 70 mg/100 ml, pH 5–5.5, body temperature 37°). The solubility of **8c** increases with increasing pH in a manner predictable from its acidity (pK' = 6.9).

Anhydrous sulfacytine (**8c**) dissolves to metastable solutions in H_2O and is more soluble than the hydrate. Such solutions saturated with the anhydrous form are stable for hours but slowly deposite the hydrate with time or when seed crystals are added. The tendency of this substance to form metastable solutions is pronounced; solutions containing sulfacytine up to many times the equilibrium solubility are notably persistent. This is depicted in Figure 1 where simple buffer solutions containing up to 0.8% sulfacytine at pH 5.9 and 37° remained clear for one to several hours. The persistence of these metastable solutions is markedly enhanced by impurities which act presumably as nucleation inhibitors. This is illustrated in Figure 1 by the dotted line detailing the persistence of an 0.8% solution of sulfacytine in human urine for 4 days. Solutions containing 1% of **8c** in human urine remained clear for only 1–2 days. Characteristically these supersaturated urine solutions, even when heavily seeded with crystalline drug, only sluggishly deposit their excess burden of compound.

Clearly because of the continuous, dynamic nature of the urine excretion process, the tendency of **8c** to form metastable solutions could be an added important safeguard against precipitation in the tubules of the kidney. This safeguard would be operative for example if the drug were overdosed or if it were inadvertently given to a severely dehydrated person. Should the excreted drug levels rise temporarily above the intrinsic solubility of the substance, the metastability of such solutions in urine could be counted on to carry

(10) E. H. Northey, "The Sulfonamides and Allied Compounds," Reinhold Publishing Co., New York, N. Y., 1948, pp 517–577.

(11) A. B. Miller, general manager, "Physicians' Desk Reference to Pharmaceutical Specialties and Biologicals," 23rd ed, Litton Publications, Inc., Oradell, N. J., 1969, p 571 for sulfamethylthiadiazole, p 1032 for sulfisoxazole.

(12) Percentage binding increases with decreasing free (or its linked total) level, see footnote to Table IV.

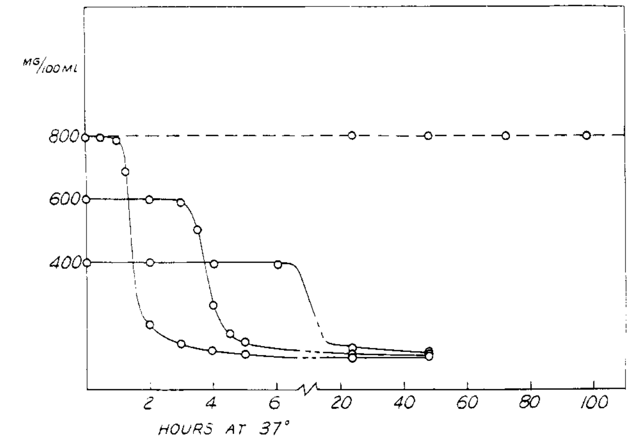


Figure 1.—Supersaturability of sulfacytine; persistence of metastable solutions: (–) in buffer pH 5.9; (– –) in urine pH 5.9.

them without crystallization further down the urinary tract or to complete excretion.

We believe (and will report later elsewhere) that inhibition of nucleation by urine constituents is part of a hitherto poorly recognized kidney protection system whereby that organ is protected from slightly soluble substances. Among the substances we have tested, sulfacytine with its inherent tendency to form metastable solutions was most affected by the nucleation inhibitors of urine.

A final observation on solubility is that N^4-acetylsulfacytine is also adequately soluble by Lehr's criteria. It equilibrates to 65 mg/100 ml in pH 5 buffer at 35°. Some sulfa drugs, themselves adequately soluble, are converted metabolically into the acetyl derivative which may be much less soluble, carrying the risk of crystalluria and its complications.[5]

In conclusion, the outstanding intrinsic antibacterial potency, high solubility, and short-acting properties of sulfacytine particularly recommend its consideration for use in urinary tract infections. Clinical studies with various drugs have shown generally that readily attained high urinary levels of active "free" drug are the principal determinants for therapeutic success.[13]

Experimental Section[14]

Microbiological Procedures.—These procedures are adequately covered in ref 4.

Physical Chemical Procedures.—The protein adsorption measurements were made after equilibrium dialysis with albumin contained in protein-impermeable cellulose film ("Visking") bags.[15,16] Care was taken to use the same lots of albumin where possible for the comparative figures given in each column of Table IV.[17] The absolute adsorption can vary for each drug depending on the particular lot of albumin used; the binding relation between drugs is maintained however.

Solubility was determined from spectrophotometric measurements on supernatant solutions; after shaking overnight the buffered suspension containing a large excess of compound. The initial metastable solutions for studies of supersaturation were obtained by heating the suspensions on a steam bath to the degree (50–90°) necessary to ensure complete solutions; these then were allowed to cool undisturbed to the desired temperature. The effects of treatments (seeding, shaking, etc.) were assessed relative to the undisturbed metastable (supersaturated) solution.

1-Alkyluracils (1).[18]—We found that hydrolysis of 1-alkyl-5-cyanouracils to the 1-alkyl-5-carboxyluracils as described by Shaw[3] was accompanied by decarboxylation and with prolonged refluxing 1-alkyluracil was the sole isolable product. Typically 2 mol of 1-alkyl-5-cyanouracil was refluxed in 3700 ml of a mixture of H_2O, concentrated aqueous HCl, and glacial HOAc (1:1:2 v/v). The product was either (A) crystallized by concentrating to small volume and separating the crystallized product by filtration, or (B) evaporating to dryness and separating from salt by extracting with solvent. The final products are listed in Table V. Isolation details follow for each compound in the order: number, hours of reflux, isolation A or B, crude yield, crude melting point: **1c**, 24, A, 82, 146–148°; **1d**, 168, A, 53, 120–121°; **1e**, 98, B (EtOH), 75, 131–133°; **1f**, 72, A, 89, 101–103°; **1g**, 50, B (EtOAc), 48, 94–95°; **1h**, 120, A, 56, 95–97°; **1j**, 98, B (EtOH), 23, —. The 1-allyl-5-cyanouracil used for **1j** is new. It was prepared by the method of Shaw,[3] mp 149–152°. *Anal.* ($C_8H_7N_3O_2$) C, H, N.

TABLE V

INTERMEDIATES

Compound	Crystn solvent	Mp, °C	Formula	*Anal.*
		1-Alkyluracils (1)		
1c	EtOH	144–146	$C_6H_8N_2O_2$	CHN
1d	*i*-PrOH	120–121	$C_7H_{10}N_2O_2$	CHN
1e	EtOH	131–133	$C_7H_{10}N_2O_2$	CHN
1f	H_2O	102–104	$C_8H_{12}N_2O_2$	CHN
1g	EtOAc	94–95	$C_8H_{12}N_2O_2$	CHN
1h	EtOAc	108–113	$C_8H_{12}N_2O_2$	CHN
1j	H_2O	105–108	$C_7H_8N_2O_2$	(CHN)[a]
		1-Alkyl-4-thiouracils (2)		
2c	MeCN	156–157	$C_6H_8N_2OS$	CHNS
2d	*i*-PrOH	89–90	$C_7H_{12}N_2OS$	CHNS
2e	EtOH	147–149	$C_7H_{10}N_2OS$	CHNS
2f	*i*-PrOH	68–70	$C_8H_{12}N_2OS$	CHNS
2g	EtOH	91–93	$C_8H_{12}N_2OS$	CHN S (calcd) 17.4 (found) 18.06
2h	EtOH	96–97	$C_8H_{12}N_2OS$	CHNS
2j	Me_2CO	122–125	$C_7H_8N_2OS$	CHNS
		1-Alkylcytosines (5)		
5c	Water	245–247	$C_6H_9N_3O$	CHN
5d	Water	256–258	$C_7H_{11}N_3O$	CHN
5e	EtOH	201–203	$C_7H_{11}N_3O$	CHN
5f	EtOH	229–231	$C_8H_{13}N_3O$	CHN
5g	MeCN	256–257	$C_8H_{13}N_3O$	CHN
5h	MeCN	217–220	$C_8H_{13}N_3O$	CHN
5j	EtOH	232–236	$C_7H_9N_3O$	(CHN)[b]
		N-(*N*-Acetylsulfanilyl)cytosines		
6a	$MeOCH_2CH_2OH$	280–282	$C_{12}H_{12}N_4O_4S \cdot H_2O$	CHNS(H_2O)
6b	H_2O–$MeOCH_2CH_2OH$	266–270	$C_{13}H_4N_4O_4S$	CHNS
6c	H_2O-$MeOCH_2CH_2OH$	238–240	$C_{14}H_{16}N_4O_4S$	CHNS
6i		242–244	$C_{19}H_{18}N_4O_4S$	CHNS

[a] Analyses corrected for 0.39% water. [b] Fits for an assumed 4.5% inert impurity. Actual analysis: Calcd: C, 55.62; H, 6.00; N, 27.80. Found: C, 53.30; H, 5.92; N, 26.44. The identity of the compound is established in a general way by its successful conversion into **7j** and **8j**.

1-Alkyl-4-thiouracils (2).—The thiation of 1-alkyluracils with P_2S_5 was performed essentially by the method of Fox, *et al.*[19]

(13) W. R. McCabe and G. G. Jackson, *New Eng. J. Med.*, **272**, 1037 (1965).

(14) Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are corrected. Where analyses are indicated only by symbols of the elements the analytical results obtained for these elements were within ±0.4% of the theoretical value.

(15) This method was detailed in a presentation by J. M. Vandenbelt at the Atlantic City Meeting of the American Drug Manufacturers Association Research and Development Section (1954).

(16) For a review of the literature on protein binding and its determination see A. Goldstein, *Pharmacol. Rev.*, **1**, 102 (1949).

(17) The human serum albumin was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.

(18) (a) The unsubstituted compound is the well-known biochemical, uracil. (b) 1-Methyluracil was described by M. R. Atkinson, M. H. Maguire, R. K. Ralph, G. Shaw, and R. N. Warrener, *J. Chem. Soc.*, 2366 (1957).

(19) J. J. Fox, D. VanPraag, I. Wempen, I. L. Doerr, L. Cheong, J. R. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Amer. Chem. Soc.*, **81**, 187 (1959).

The products were isolated either by (A) crystallizing the crude product from EtOH, (B) EtOH–H_2O crystallization, or (C) extraction with dilute NH_3 and subsequent acidification. The final products are listed in Table V. Isolation details follow for each compound in the order number, isolation procedure, crude yield, crude melting point (**2a** and **2b** are known[19,20]): **2c**, A, 61, 154–157°; **2d**, A, 51, 87–88°; **2e**, A, 76, 145–148°; **2f**, B, 72, 66–69°; **2g**, C, (27% for twice crystallized product, mp 91–93°); **2h**, A, 53, 94–95°; **2j**, C, 76, 90–117°.

1-Benzyl-4-(methylthio)-2(1*H*)-pyrimidinone (4i).—To a soln of NaOMe from 2.3 g of Na in 200 ml of MeOH was added 14.2 g (0.1 mol) of *S*-methyl-4-thiouracil[20] (**3**). To the stirred solution was added 18.8 g (0.11 mol) of $PhCH_2Br$ and it was refluxed for 10 min. The now neutral solution was evaporated to dryness and the product extracted ($CHCl_3$) and crystallized by adding several volumes of Et_2O and cooling: yield, 21 g (91%) crude **4i**, mp 132–139°, crystallized from MeCN, then H_2O, mp 151–152°. *Anal.* ($C_{12}H_{12}N_2OS$) C, H, N, S.

1-Ethyl-4-(methylthio)-2-(2*H*)-pyrimidinone (4c).—In dilute alkali **3** was treated with $(EtO)_2SO_2$ and the product isolated as above: crude semisolid from $CHCl_3$, 93% yield; crystallized from Et_2O then CCl_4, mp 66–67°. *Anal.* ($C_7H_{10}N_2OS$) C, H, N, S.

1-Alkylcytosines (5).—The various 1-alkyl-4-thiouracils (**2**) were treated with alcoholic NH_3 at 120° for 24 hr.[19] Typically, 0.2 mol of thiouracil was treated with 300 g of NH_3 in 1.2 l. of MeOH in a rocking autoclave under endogenous pressure. Simple cooling of the reaction mixture either directly or after concentrating to a small volume gave the following yields of crude products: **5c** (1-Et), 43%, mp 242–245°; **5d** (1-Pr), 56% (final crystallization from H_2O), 256–258°; **5e** (1-*i*-Pr), 48%, mp 198–201°; **5f** (1-Bu), 74%, mp 226–228°; **5g** (1-*i*-Bu), 58%, mp 250–253°, 2nd crop 242–245°; **5h** (1-*sec*-Bu) purified by way of hydrochloride (mp 223–251°) converted into free base with NH_3, mp 214–217°; **5j** (1-allyl), 49% (from EtOH), mp 229–233°. The purified final products are listed in Table V.

***N*-(*N*-Acetylsulfanilyl)cytosines (6) by Fusion of 4-(Methylthio)-2(1*H*)-pyrimidinones (4) with *N*[4]-Acetylsulfanilamide.**—Equimolar quantities of reactants[20] (typically 0.1 mol of each) were fused under N_2 at *ca.* 205–210° until MeSH evolution had practically ceased (30–60 min). The bath was removed and to the slightly cooled but still molten mass was added *ca.* 1 volume of solvent. The crude product crystallized and was collected after cooling. Using the named solvent the following products, melting points, and yields were obtained: **6a**, EtOH, mp 230–268°, 98%; **6b**, EtOH, mp 255–263°, quant.; **6c**, $MeOCH_2CH_2OH$–H_2O, 239–256° (crude), 73%; **6i**, EtOH, 242–244°, 68%. The purified products are listed in Table V.

***N*-(*N*-Acetylsulfanilyl)-1-ethylcytosine (6c) from *N*-Acetylsulfanilyl Chloride and 1-Ethylcytosine (5c).**—In 122 ml of MeCN were suspended 11.7 g (0.05 mol) of acetylsulfanilyl chloride and 7.0 g (0.05 mol) of 1-ethylcytosine. After adding 7.4 ml of Et_3N the suspension was refluxed for 16 hr; dissolution was complete after 1 hr. The solution was cooled to crystallize the product which was filtered off and washed (dil acid, H_2O, EtOH): yield, 8.36 g (50%); mp 237–239° dec; 288 mμ, $E_1{}^1$ = 519; 261 mμ, $E_1{}^1$ = 590 in pH 7 buffer; (for above analyzed sample, from fusion, mp 238–240°; λ_{max} 287 mμ, $E_1{}^1$ = 562; λ_{max} 261 mμ, $E_1{}^1$ = 638).

***N*-(*p*-Nitrophenylsulfonyl)cytosines (7).**—These compounds were prepared by treating the appropriate cytosine **5** in pyridine at 55–65° with *p*-nitrobenzenesulfonyl chloride. The low solubility and low reactivity of most of the cytosines **5** made some changes necessary. Typically 0.2 mol of the cytosine was suspended in 2 l. of pyridine and heated (even to reflux) to dissolve the substituted cytosine if possible. The solution (or suspension) was then cooled quickly to *ca.* 65° and held at 55–65° during the slow addition of *p*-nitrobenzenesulfonyl chloride and for 4–6 hr. The product was isolated, after evaporating the excess pyridine under reduced pressure, by quenching the residual syrup in cold dilute acid. The products were purified by dissolving them in dilute alkali or NH_3, treating with charcoal, filtering, and acidifying. These sulfonamides were analyzed and used in the reduction step usually without further purification. The following yields, melting points, and analyses were obtained: **7c** (35% crude), 186° dec (from MeCN) ($C_{12}H_{12}N_4O_5S$); *Anal.* C, H, N, S; **7d** (37% crude, mp 147–158°), 159–161° (MeCN) ($C_{13}H_{14}N_4O_5S$); *Anal.* C, H, N; **7e**, 66%, 194–203°, ($C_{13}H_{14}N_4O_5S$) *Anal.* C, H, N; **7g**, 34%, 175–178° ($C_{14}H_{16}N_4O_5S$); *Anal.* C, H, N; **7h** (reprecipitated), 47%, 197–199°, ($C_{14}H_{16}N_4O_5S$); *Anal.* C, H, N; **7j**, 42%, 162–166° ($C_{13}H_{12}N_4O_5S \cdot 0.5H_2O$); *Anal.* C, H, N.

Sulfa Drugs by Fe–Dilute AcOH Reduction of Intermediates 7. These crude derivatives are listed in Table VI prepared following the procedure used for **8c**. The final crystallized products are listed in Table I.

***N*-Sulfanilyl-1-ethylcytosine [*N*[1]-(1-Ethyl-1,2-dihydro-2-oxo-4-pyrimidinyl)sulfanilamide] (8c).**—Compound **7c** was reduced in Fe–H_2O suspension containing a trace of HOAc. The suspension after adding an equal volume of EtOH and excess NH_4OH was filtered. The filtrate was concentrated to small volume under reduced pressure and acidified with HOAc to *ca.* pH 6. The gummy precipitate solidified; yield 85%, mp 95–98° (hydrate); crystallized successively from BuOH, H_2O, and MeOH (melting point etc., see Table I) (λ_{max} 297 mμ, $E_1{}^1$ = 762; λ_{max} 263 mμ, $E_1{}^1$ = 584, in MeOH).

Hydrate.—Another lot of anhydrous material **8c** (mp 167–168°, λ_{max} 298 mμ, $E_1{}^1$ = 755; 253 mμ, $E_1{}^1$ = 580, in MeOH) was dissolved in dilute alkali and precipitated slowly by acidifying with HOAc with seeding and scratching, recovery quantitative, (melting point etc. see Table I), λ_{max} 298 mμ, $E_1{}^1$ = 705; 263 mμ, $E_1{}^1$ = 542 (MeOH).

Table VI

Compound	Compound reduced[a]	Crude yield, %	Mp, °C	Crystn Solvent
8b	7b	68	190–192	EtOH
8d	7d	74	88–131	MeCN
8e	7e	84	202–204	EtOH
8f	7f	62	95–100	EtOH
8g	7g	91		MeCN[b]
8h	7h	96	132–137	MeOH
8j	7j	83	158–165	MeCN

[a] Reduced by the same method as **7c**. [b] Crystallization yield 62%.

***N*-Sulfanilycytosines (8) by Alkali Hydrolysis of Acetyl Derivatives (6).**—Typically, 0.1 mol of the acetyl derivatives **6** was refluxed for 1 hr in 200 ml of 2 *N* NaOH. The cooled solution was then acidified with HOAc to *ca.* pH 5 and the gummy precipitate crystallized gradually. The product was filtered off and air-dried; it was then crystallized for analysis or comparison with earlier preparations. See Table I for final melting points. The following crude yields and the melting points were obtained: **8a** (R = H), 98%, 277–279° (from 1:1 $MeOCH_2CH_2OH$–H_2O for analysis); **8b**, 87%, mp 170–210° (from MeCN, then EtOH, for analysis); **8c**, 77%, mp 106–145° (successively from EtOH, MeCN, BuOH, and MeOH, mp 167–169°); **8i**, 93%, mp 197–201° (from 1:1 MeOH–H_2O) and dried for 6 hr at 100° for analysis.

Acknowledgments.—We are indebted to Mr. Charles E. Childs and associates for the microanalyses reported here. We are pleased to acknowledge the competent assistance of Mrs. Carola H. Spurlock in the spectral, solubility, and protein binding measurements.

(20) The *S*-methyl-4-thiouracil and 1,*S*-dimethyl-4-thiouracil are described by H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, **42**, 30 (1909).